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Prophylaxis of acute respiratory virus infections using nucleic acid-based drugs

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Abstract

Acute respiratory virus infections such as SARS and pandemic influenza are highly contagious diseases that cause global crisis, and inflict severe human mortality and morbidity. Vaccines against these viruses are either unavailable or do not provide adequate protection. In the absence of effective vaccines, nucleic acid-based immunomodulators have the potential to offer effective, broad-spectrum protection against these deadly pathogens. Poly ICLC and CpG oligonucleotides are promising gene-based drugs which have been shown in animal studies to protect against acute respiratory virus infections. Poly ICLC is a synthetic double-stranded RNA (dsRNA), and an effective interferon-inducer and natural killer cell activator. When encapsulated in liposomes, poly ICLC offers complete protection (100% survival rate in pretreated group versus 0% survival in control group) against a lethal respiratory challenge of influenza A virus in mice. This antiviral effect has been shown to persist for up to 3 weeks post-drug treatment. Poly ICLC pretreatment also protects mice against a respiratory challenge of western equine encephalitis (WEE) virus, at a level comparable to inactivated WEE vaccine. CpG oligos in liposomes also provided high level of protection against the lethal influenza challenge. Together, these studies suggest nucleic acid-based immunomodulators are promising antiviral agents which can offer effective and non-specific protection against acute respiratory virus infections.

Keywords: Nucleic acids; Protection; Viral infections

1. Introduction

Acute respiratory virus infections such as SARS and avian influenza are zoonotic diseases that have the potential to cause devastating global pandemics. These viral diseases exert an enormous toll on human lives and global economy, as exemplified by the Spanish flu of 1918–1919, which caused more than 25–40 million human lives worldwide [1]. The viruses that cause these diseases can also be potentially weaponized as bioterrorism and/or biological warfare agents by hostile forces [2].

Vaccines are considered to be the most effective countermeasures [3], but licensed vaccines against these viruses are either not available, or the efficacies have yet to be documented. The development of novel antiviral drugs is a positive development in the treatment of these viral diseases. The major limitations in the therapeutic applications of antiviral drugs against deadly diseases such as SARS and bird flu are availability and drug resistance [4]. Since outbreaks and pandemics can occur anytime globally, and current vaccines are not available, alternate prophylactic and therapeutic measures, which are safe and effective will need to be evaluated and developed quickly. Of particular value are antiviral agents that may elicit long-lasting broad-spectrum protective immune responses to a range of respiratory virus pathogens.

Nucleic acid-based drugs and vaccines are potentially promising classes of antiviral agents that have important applications against acute respiratory virus infections. Nucleic acids can either provide highly specific antiviral activity

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against specific strains of viruses, or elicit broad-spectrum antiviral activity against a wide range of viruses. Nucleic acid-based drugs, which stimulate host's immune defense responses offer a significant therapeutic advantage in that they can offer protective antiviral activity against a wide range of viruses, regardless of genetic mutations, recombinations, or zoonotic origin.

There are currently two major classes of nucleic acid-based drugs, which are effective in eliciting broad-spectrum protective antiviral activity, namely poly ICLC, a synthetic RNA [5,6], and CpG oligonucleotides, RNA/DNA sequences that contain unmethylated CpG motifs [7]. One major drawback of using nucleic acid-based antiviral agents is that the susceptibility to nuclease degradations in vivo. Chemical modifications to the nucleic acid backbones or sugars to enhance nuclease-resistance; however, these chemical modifications have been correlated with increased toxicity [8]. Liposome drug delivery systems have been evaluated for the ability to protect nucleic acid-based drugs against nuclease degradation, as well as to reduce their intrinsic toxicity in vivo [9,10]. In this paper, we highlight the recent development of poly ICLC and CpG oligonucleotides against respiratory viral infections, including influenza A virus, and demonstrate the therapeutic advantages of using liposomes as delivery systems for DNA/RNA. Nucleic acid-based drugs may have a significant role to play in medical countermeasures against pandemic and emerging viral diseases.

1.1. The antiviral efficacies of poly ICLC and liposomal poly ICLC

Poly ICLC is a synthetic double-stranded polyriboinosinic–polyribocytidylic acid (poly IC) stabilized with poly-L-lysine:carboxymethyl cellulose (LC). It has been shown to be a potent immunomodulating agent [5,6]. Poly ICLC has been shown in animal studies to be effective in providing protection against a range of infectious viruses including those causing yellow fever, Rift Valley fever, rabies and Venezuelan equine encephalomyelitis. The antiviral activity of this complex is believed to be mediated by the ability of double-stranded (ds) RNA to stimulate the production of α, β, and γ-interferons in vivo and to stimulate specific components of the cellular and humoral immune systems, including the activation of natural killer cells [5].

1.1.1. Efficacy against influenza A virus infection

The antiviral efficacy of poly ICLC was determined using a lethal respiratory influenza virus model in mice. Intranasal (IN) pretreatment with poly ICLC (1 mg/kg body weight) provided complete protection against both influenza A/Aichi/2 (H3N2) and influenza A/PR/8 (H1N1) [6]. A liposome formulation for the encapsulation of poly ICLC was developed [10]. When the antiviral activity and toxicological profiles of poly ICLC and liposomal poly ICLC were compared in the mice, a number of therapeutic characteristics were observed. Liposome encapsulation of poly ICLC prolonged the window of protection from 14 days for poly ICLC to 21 days [10]. When mice were pretreated with liposomal poly ICLC within 21 days prior to virus challenge, they were completely protected from the virus challenge, while untreated mice succumbed to the lethal influenza virus challenge [10]. When the toxicity profiles of the free and liposomal poly ICLC were compared in mice, it was found that hypothermia and body weight loss induced by poly ICLC were either completely mitigated or significantly reduced in mice given equivalent and therapeutically active doses of poly ICLC in the liposomal form [10]. A small pilot study to evaluate poly ICLC and liposomal poly ICLC against the avian influenza virus is currently being planned.

Table 1
Prophylactic efficacy of poly ICLC and liposomal poly ICLC against respiratory WEE infection in mice

Pretreatment	Route	# Survivors/ total	% Survival	p vs. control
Untreated control (PBS)	IM	0/5	0	–
Poly ICLC	IM	5/5	100	<0.05
	IN	5/5	100	<0.05
Liposomal poly ICLC	IM	5/5	100	<0.05
	IN	4/4	100	<0.05
Inactivated WEE vaccine	IM	7/8	88	<0.05
	IN	ND		

ND: not determined.

1.1.2. Efficacy against western equine encephalitis virus

The efficacy of poly ICLC and liposomal poly ICLC to protect against a lethal respiratory challenge of multiple lethal doses of western equine encephalitis (WEE, Fleming strain) virus in mice was evaluated (Table 1). Both free and liposome-encapsulated poly ICLC (1 mg/kg body weight) administered intramuscularly (IM) provided complete protection to mice against lethal WEE challenge, while all infected control mice succumbed to the infection ($p < 0.05$ versus unpretreated control group). This level of protection provided by either poly ICLC or liposomal poly ICLC administered IM or IN was found to be comparable or higher than that provided by inactivated WEE vaccine.

1.2. The antiviral efficacy of CpG oligonucleotides

CpG oligonucleotides RNA/DNA or oligonucleotides containing unmethylated CpG motifs have been shown to have strong immunostimulatory properties [7]. The effects of CpG DNA on the immune systems are very diverse; including B-cells proliferation, activation of macrophages, monocytes, dendritic cells and natural killer cells. The activation of these cells also results in the induction of various cytokines including IFN-α, β, γ, IL-6, IL-12, GM-CSF,

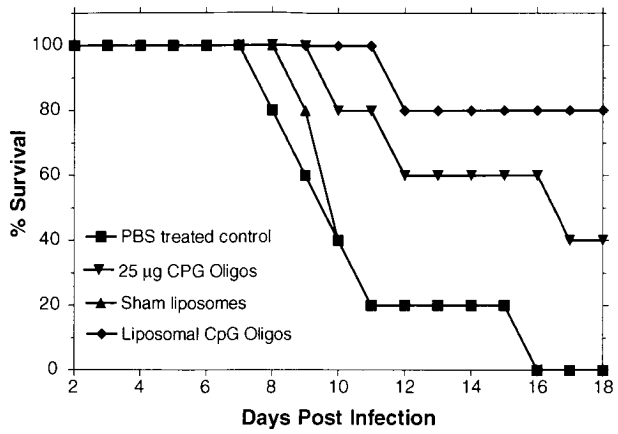

Fig. 1. Prophylactic efficacy of CpG oligos against respiratory influenza virus infection. Groups of mice were pretreated with one single IN dose of 25 μg of CpG oligos or CpG oligos in liposomes. At 5 days post-treatment, mice were challenged with 10 LD_{50} influenza A virus, and the survival rates were determined.

and TNF-α. The ability of CpG DNA to induce systemic (humoral and cellular) immune responses as well mucosal immunity at local and distant sites without causing significant toxicity render CpG DNA an ideal candidate as an immunomodulator against virus infections [7]. Indeed, CpG DNA has been shown to be an excellent adjuvant for enhancing immunological responses against a number of vaccine candidates including influenza, hepatitis, HIV, and herpes viruses.

This present study evaluates whether CpG oligonucleotide as a stand-alone immunomodulator can induce protective antiviral immunity against influenza A virus infection. Using the lethal murine influenza A virus model described above [6], the prophylactic efficacy of CpG oligonucleotides against 10 LD_{50} of influenza virus was evaluated (Fig. 1). Preliminary results indicated that one single intranasal dose of 25 μg CpG oligonucleotides given at 5 days prior to virus challenge provided partial protection to mice against the virus infection. When the CpG oligonucleotide was formulated in liposomes, the antiviral efficacy increased from 40% to 80% ($p < 0.05$ versus control). Current efforts are directed at optimizing the dosing regimen and improving liposome formulation, which can significantly enhance the antiviral efficacy of CpG oligonucleotides against influenza and other acute respiratory virus infections.

2. Conclusion

The results presented suggest that nucleic acid-based drugs such as poly ICLC and CpG oligonucleotides have the ability to elicit protective broad-spectrum antiviral immunity against a number of pathogenic viruses. Liposomes are promising carrier system for nucleic acid-based drugs by protecting them against in vivo nuclease degradation, delivering them to intracellular sites of infection, and reducing intrinsic drug toxicity [9,10]. Liposome formulated nucleic acid-based drugs may have a significant role to play in the medical countermeasures against acute respiratory virus infections such as SARS and avian influenza.

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